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The prevalence of chromosomal abnormalities in subgroups of infertile men[†]

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BACKGROUND: The prevalence of chromosomal abnormalities is assumed to be higher in infertile men and inversely correlated with sperm concentration. Although guidelines advise karyotyping infertile men, karyotyping is costly, therefore it would be of benefit to identify men with the highest risk of chromosomal abnormalities, possibly by using parameters other than sperm concentration. The aim of this study was to evaluate several clinical parameters in azoospermic and non-azoospermic men, in order to assess the prevalence of chromosomal abnormalities in different subgroups of infertile men.

METHODS: In a retrospective cohort of 1223 azoospermic men and men eligible for ICSI treatment, we studied sperm parameters, hormone levels and medical history for an association with chromosomal abnormalities.

RESULTS: The prevalence of chromosomal abnormalities in the cohort was 3.1%. No association was found between chromosomal abnormalities and sperm volume, concentration, progressive motility or total motile sperm count. Azoospermia was significantly associated with the presence of a chromosomal abnormality [15.2%, odds ratio (OR) 7.70, $P < 0.001$]. High gonadotrophin levels were also associated with an increased prevalence of chromosomal abnormalities (OR 2.96, $P = 0.013$). Azoospermic men with a positive andrologic history had a lower prevalence of chromosomal abnormalities than azoospermic men with an uneventful history (OR 0.28, $P = 0.047$). In non-azoospermic men, we found that none of the studied variables were associated with the prevalence of chromosomal abnormalities.

CONCLUSIONS: We show that the highest prevalence of chromosomal abnormalities is found in hypergonadotrophic azoospermic men with an uneventful andrologic history.

Key words: chromosomal abnormalities / male infertility / azoospermia / ICSI

Introduction

In men with poor sperm quality, the prevalence of chromosomal abnormalities is assumed to be higher than in the general population (first reported by Chandley *et al.*, 1975). Some chromosomal abnormalities, such as Klinefelter's syndrome and Robertsonian translocations, are associated with infertility. Paternal chromosomal abnormalities may increase the risk of recurrent miscarriages or a child with congenital anomalies. As a consequence, the introduction of ICSI as a means for men with poor sperm quality to produce offspring has caused concerns of increasing transmission of the

chromosomal abnormality to the progeny. Guidelines state that screening for chromosomal abnormalities is a prerequisite before ICSI treatment in the case of (severe) male infertility (NVOG, 1999; Crosignani and Rubin, 2000; Foresta *et al.*, 2002; NICE, 2004; AUA and ASRM, 2006).

The reported prevalence of chromosomal abnormalities in infertile men ranges from 3 to 19% (summarized by Martin, 2008), depending on the population studied. In some studies, all male partners of infertile couples were karyotyped, while other studies only did so for men with extremely poor sperm quality. Because karyotyping is costly and time-consuming, it would be of benefit to identify those infertile men

[†]This study was partly presented as a poster at the ESHRE Annual meeting, Stockholm, 2011.

who have the highest risk of carrying a chromosomal abnormality. So far, few studies have taken into account sperm parameters and patient factors of infertile men as possible risk factors for chromosomal abnormalities.

Some studies on sperm parameters have indicated that sperm concentration shows the best correlation with the presence of chromosomal abnormalities (Chandley *et al.*, 1975; Chandley, 1979; De Braekeleer and Dao, 1991; Bourrouillou *et al.*, 1992; Van Assche *et al.*, 1996; Gekas *et al.*, 2001; Clementini *et al.*, 2005). These studies report that with decreasing sperm concentration, the prevalence of chromosomal abnormalities increases, with most aberrations found in azoospermic men (reviewed in Dul *et al.*, 2010). However, other studies could not confirm this linear correlation in subgroups of infertile men (van der Ven *et al.*, 1997; Yoshida *et al.*, 1997; Riccaboni *et al.*, 2008; Dul *et al.*, 2010). These conflicting results seem to be caused by differences in the study populations (e.g. whether the couples or the men were infertile) and in categorization of sperm concentrations.

Sperm motility and morphology have also been studied in relation to chromosomal abnormalities. In several studies, no difference in these sperm parameters was found between men with normal and abnormal karyotypes (Marmor *et al.*, 1980; Matsuda *et al.*, 1991; Yoshida *et al.*, 1997; Gekas *et al.*, 2001). Some studies did find a significant difference, but only in oligozoospermic men (Chandley *et al.*, 1975; Van Assche *et al.*, 1996). Yoshida *et al.* (1997) found a significantly higher incidence of chromosomal abnormalities in men with a low total motile sperm count (TMSC). The results of the studies on sperm motility and morphology are therefore inconsistent and sperm concentration is a confounder that has not been taken into account in all studies.

Few studies on chromosomal abnormalities in infertile men have focused on patient characteristics. No studies have been performed on whether a positive family history for infertile (male) relatives, relatives with recurrent miscarriages or children with multiple congenital anomalies or mental retardation is a good predictor for chromosomal abnormalities in infertile men. An increased prevalence of chromosomal abnormalities has been reported in men with testicular atrophy, high levels of FSH or high levels of LH (Yoshida *et al.*, 1997). Other studies excluded men with these characteristics, or found no differences in gonadotrophin levels between men with or without chromosomal abnormalities (Haidl *et al.*, 2000).

Most studies on the prevalence of chromosomal abnormalities have focused on men with 'unexplained' infertility, excluding men with a history of cryptorchidism, varicocele, radiotherapy, chemotherapy or previous surgery. These characteristics from a man's medical history might be causally related to infertility, but it is also possible that infertility and the characteristic have a common genetic origin. In the testicular dysgenesis syndrome an association has been suggested between cryptorchidism, poor sperm quality and genetic abnormalities (Akre and Richiardi, 2009). It has not been established whether there is an independent correlation between these patient characteristics and chromosomal abnormalities.

So far, sperm concentration is the most frequently studied parameter in relation to the prevalence of chromosomal abnormalities in infertile men, and there have been no systematic studies on correlations with other sperm parameters, hormone levels and patient characteristics. In a previous study, we found a significant difference in the

prevalence of chromosomal abnormalities between azoospermic and non-azoospermic men, and in non-azoospermic men sperm concentration was not correlated with chromosomal abnormalities (Dul *et al.*, 2010). The aim of the present study is to evaluate additional sperm parameters and patient characteristics in azoospermic and non-azoospermic men, in order to assess the prevalence of chromosomal abnormalities in different subgroups of infertile men.

Materials and Methods

We performed a retrospective cohort study in unselected male partners of consecutive couples applying for ICSI and azoospermic men attending our fertility clinic between November 1994 and October 2007. Couples were eligible for ICSI in the case of severe male factor infertility (defined by TMSC < 4 million in repeated semen analyses, or when the yield of a sperm preparation procedure with density-gradient centrifugation followed by swim-up was < 0.5 million motile spermatozoa), or in the case of total fertilization failure in a previous IVF procedure. These men were karyotyped in accordance with the prevailing guideline of the Dutch Society of Obstetrics and Gynaecology (NVOG, 1999). Before intake, all men received a comprehensive questionnaire on duration of infertility, previous pregnancies and their outcomes, andrologic history and family history regarding infertility, recurrent miscarriages and children with congenital abnormalities or mental retardation.

Men whose results of the chromosomal analysis and at least one sperm analysis were available were included in the study. Data were collected by chart review. In the Netherlands, no ethical board approval is required for retrospective chart review and collection of anonymized data. Couples attending our fertility clinic are informed at intake about the possible use of their anonymized data for research purposes, and a 'no objection procedure' is followed. Only patients who had not objected were included in the present study.

Chromosomal analysis

Chromosomal analysis was performed on cultured peripheral lymphocytes. Five Giemsa-Trypsin-Giemsa-banded metaphase spreads with a minimal banding resolution of 550 were analysed per patient. In the case of numerical mosaics, 100 metaphases were examined by conventional microscopic screening or fluorescence *in situ* hybridization (FISH). If structural chromosomal aberrations were present, the evaluation was extended to additional molecular cytogenetic analysis by FISH or array comparative genome hybridization, whenever appropriate. Chromosomal heteromorphisms, as defined in the 2009 International System for Human Cytogenetic Nomenclature, were not considered as chromosomal abnormalities (Shaffer *et al.*, 2009).

Sperm analysis

Sperm analyses were carried out according to the WHO criteria (WHO, 1999). Semen samples were analysed within 1 h following ejaculation by using a computer-aided semen analyzer (Strömberg Mika-cell motility analyzer; Medical Technologies Montreux SA, Montreux, Switzerland) (Togni *et al.*, 1995). If the sperm concentration was < 3 million/ml, standard microscopic investigation was performed. Semen volume, sperm concentration and progressive motility (a + b quality), and TMSC were recorded. Sperm samples directly collected in a medium were excluded, as well as sperm analyses culminating in preparation procedures for fertility treatments. All consecutive sperm analyses up to a maximum of six were included in the study. Only data from the first sperm analysis were used for the statistical analyses.

Hormone analysis

Serum FSH and LH levels were measured by fluoroimmuno-metric determination on the AutoDelfia (Wallac/Perkin Elmer, Turku, Finland). Serum total testosterone was measured by in-house radio-immunoassay, using (1,2,6,7³H)-testosterone as tracer (Amersham Biosciences, Buckinghamshire, UK) (Pratt et al., 1975). Cut-off levels were chosen based on reference values in our laboratory, as well as on cut-off levels mentioned in studies on hormone levels and Y chromosome microdeletions (Pieri et al., 2002; Kunej et al., 2003; Vutyavanich et al., 2007; Wang et al., 2010).

Statistical analysis

Statistical analysis was carried out using the Statistical Package for the Social Sciences version 18.0 for Windows (SPSS Inc., Chicago, IL, USA). Data were described in absolute counts and proportions. Associations were assessed by univariate binary logistic regression. Subsequently, a multivariate binary logistic regression analysis was performed with forward stepwise conditional inclusion of variables. At each step, the variable with the next-lowest *P*-value was included. The maximum *P*-value for inclusion was 0.4. Odds ratios (ORs), 95% confidence interval (CI) and *P*-values are presented. In the case of categorical variables, the OR represents the change in risk of carrying a chromosomal abnormality compared with the reference category, as indicated in the results. For dichotomous (yes/no) variables, the reference category was 'no'. Results were considered statistically significant when *P* < 0.05.

Results

The results of the chromosomal analysis and of at least one in-house sperm analysis were available for 1223 men with azoospermia or applying for ICSI. The median male age was 34.6 years (range 22.0–63.6) and the couples had a median duration of infertility of 2.9 years (range 0–17.6) at intake in our centre. A primary infertility was present in 85.5% of the couples. In two couples (0.16%), it was unknown whether they had had a previous pregnancy.

A chromosomal abnormality was found in 38 men (3.1%). These abnormalities consisted of 19 gonosomal aberrations: five 47,XXY, two 47,XXY/46,XY mosaics, three 47,XYY, one 47,XYY/46,XY mosaic, three 45,X/46,XY mosaics, one 46,XX and four complex aberrations of the X or Y chromosome. There were 19 autosomal aberrations: five Robertsonian translocations, six reciprocal translocations and seven inversions and one individual had a Robertsonian translocation as well as an inversion.

For all 1223 men, at least one sperm analysis was recorded. In 956 men (78.2%) at least two and in 558 men (45.6%) three or more sperm analyses were available. The outcome of the binary logistic analyses of the parameters of the first sperm analysis is shown in Table I. Median sperm volume was 3.8 ml (range 0.1–15.1), median sperm concentration was 5.0 million/ml (0–185), median progressive motility was 18% (0–100) and median TMSC was 2.184 million (0–466.2). Of the sperm parameters studied, only sperm motility had a statistically significant association with the risk of carrying a chromosomal abnormality (OR 1.02). Sperm concentration and TMSC as continuous variables showed no association with chromosomal abnormalities. Only azoospermia was significantly associated with the risk of carrying a chromosomal abnormality (OR 7.70). As shown previously, the prevalence of chromosomal abnormalities in non-azoospermic men did not differ significantly between the different categories of sperm concentration (Dul et al., 2010). In 10/12 azoospermic men with an

aberrant karyotype, a gonosomal abnormality was found (83.3%), while autosomal aberrations predominated in the non-azoospermic men with an abnormal karyotype (17/26; 65.4%).

Table I also gives the results of the univariate regression analysis of the patient characteristics. Hormone levels were not determined in all patients. FSH was determined in 86.1% of azoospermic men and in 40.6% of non-azoospermic men. The combination of FSH and LH was determined in 65.8% of azoospermic men and in 22.6% of non-azoospermic men. Of the total cohort of 1223 men, a result for serum gonadotrophins was available in 534 men (43.7%). Total testosterone was determined in 81.0% of men with azoospermia and 33.9% of non-azoospermic men. Analysis of these data showed that men with FSH > 10 IU/l or LH > 12 IU/l had an increased risk of a chromosomal abnormality compared with men with normal gonadotrophin levels (OR 2.94 and 9.42, respectively).

Compared with men whose partners had no previous pregnancy, men who had achieved conception before, irrespective of its outcome, had a lower risk of a chromosomal abnormality (OR 0.21). Total fertilization failure (TFF) in previous IVF treatment did not show an association with chromosomal abnormalities. A history of cryptorchidism or genital surgery did not influence the risk for chromosomal abnormalities, nor did a positive family history for infertility, recurrent miscarriage or children with congenital anomalies. However, not all questionnaires were filled in completely and there were data missing on several aspects of the medical history in our cohort.

There was a significant difference in the frequency of chromosomal abnormalities between azoospermic and non-azoospermic men, and therefore a univariate regression analysis for the various variables was performed in both groups. Table II gives the results for the 79 azoospermic men in our cohort, and shows a positive association between FSH and LH concentrations and chromosomal abnormalities. LH > 12 IU/l significantly increased the chance of finding a chromosomal abnormality (OR 6.83), but the increased risk in men with FSH > 10 IU/l was not statistically significant (OR 4.05). Azoospermic men with a positive andrologic history (e.g. genital infection, chemotherapy, radiotherapy, vasectomy, testicular torsion, varicocele and cryptorchidism) had a lower prevalence of chromosomal abnormalities (OR 0.28) compared with men with an uneventful history.

Table III gives the results for the 1144 non-azoospermic men in our cohort. No association was found between chromosomal abnormalities and serum hormone levels, previous pregnancy, family history or cryptorchidism. In this subgroup of men, no statistically significant association was found between chromosomal abnormalities and a positive andrologic history.

Discussion

We found that the prevalence of chromosomal abnormalities was not associated with any sperm parameter besides azoospermia, whereas high gonadotrophin levels and a negative andrologic history were associated with an abnormal karyotype.

Our study is based on data from a large cohort of infertile men who were either azoospermic or eligible for ICSI, representing an unselected population of males visiting a tertiary referral centre. The prevalence of chromosomal abnormalities found in our cohort was 3.1% (CI 2.1–4.1), which is in the lowest range of the 3–19% mentioned in

Table 1 Sperm parameters and patient characteristics in a cohort of 1223 men with azoospermia or applying for ICSI in relation to chromosomal abnormalities.

Characteristic	n	Abnormal karyotype (38)	Normal karyotype (1185)	Univariate analysis		
				OR	95% CI	P-value
Sperm volume per ml	1223	3.6 (0.2–13.9)	3.7 (0.1–15.1)	1.04	0.885–1.21	0.660
Sperm concentration per million/ml	1223	0.209 (0–40.0)	5.000 (0–185)	0.975	0.942–1.01	0.148
Azoospermia	79	15.2%	84.8%	7.70	3.72–15.9	<0.001
Non-azoospermia	1144	2.3%	97.7%	1.0		
Sperm motility per %	1144	26.0 (0–100)	18.0 (0–100)	1.020	1.003–1.018	0.024
TMSC per million	1223	0.1755 (0–38.5)	2.2000 (0–466.2)	0.984	0.952–1.02	0.345
Previous pregnancy by male partner	1214	5.3%	20.6%	0.214	0.051–0.827	0.035
Previous miscarriage(s)	244	50.0%	34.3%	1.92	0.118–31.0	0.647
ICSI because of TFF	1223	10.5%	15.3%	0.653	0.229–1.86	0.425
FSH per IU/l	532	19.0 (3.33–48.4)	7.00 (0.19–49.8)	1.07	1.04–1.11	<0.001
FSH > 10 IU/l	532	60.9%	34.6%	2.94	1.25–7.27	0.014
LH per IU/l	262	6.57 (1–25.3)	3.90 (0.09–25.1)	1.20	1.09–1.33	<0.001
LH > 12 IU/l	262	17.6%	2.4%	9.42	2.12–42.0	0.003
Increased gonadotrophins ^a	534	60.9%	34.4%	2.96	1.26–6.98	0.013
Testosterone per nmol/l	452	15.50 (3.3–25)	16.55 (3.45–51)	0.947	0.880–1.02	0.148
Positive andrologic history ^b	1191	36.8%	50.6%	0.570	0.292–1.11	0.100
Positive family history ^c	1117	22.2%	18.6%	1.25	0.562–2.79	0.584
History of cryptorchidism	1190	23.7%	20.5%	1.21	0.563–2.58	0.632

Data are median (range) or prevalence.

n Number of cases of which data were available for analysis.

TMSC, total motile sperm count; FSH, follicle-stimulating hormone; LH, luteinizing hormone; TFF, total fertilization failure in previous IVF.

^aIncreased gonadotrophins: FSH > 10 IU/l and/or LH > 12 IU/l.

^bPositive andrologic history: cryptorchidism (245), genital infection (94), vasectomy (33), orchidectomy (49), radiotherapy (9), chemotherapy (18), testicular biopsy (31), hypospadias or midline defect (8), varicocele (122), spermatocele (5), surgery for hydrocele (8), surgery of urethra or ejaculatory duct (9), surgery for inguinal hernia (121), accidental testicular injury (29), testicular torsion (23), congenital anomaly of urogenital tract (13), exposition to diethylstilbestrol *in utero* (4). Note: several events may have occurred in one case.

^cPositive family history: (male) relatives with infertility (144), recurrent miscarriages (49), children with congenital anomalies or mental retardation (34).

published studies (summarized by Dul *et al.*, 2010). This may be explained by the wide range in sperm quality in our cohort, as we included azoospermic males as well as men with normozoospermia who had TFF in a previous IVF treatment. This allowed us to compare groups of men with different sperm qualities in relation to chromosomal abnormalities.

The chromosomal abnormalities we found are comparable with those reported in other studies in infertile men: gonosomal aberrations are most frequently detected in men with azoospermia, while autosomal abnormalities, e.g. translocations are the most frequently reported aberrations in non-azoospermic men (Dohle *et al.*, 2007; O'Flynn O'Brien *et al.*, 2010). The association of gonosomal aberrations, in particular Klinefelter's syndrome, and primary testicular failure is well known (Forti *et al.*, 2010). Balanced structural chromosome aberrations seldom result in azoospermia; they usually present with a phenotype varying from severe oligozoospermia to normozoospermia.

We performed our statistical analyses on the basis of the first sperm analysis recorded in our centre. There is a large within-subject variability in consecutive sperm analyses (Keel, 2006; Francavilla *et al.*, 2007; Leushuis *et al.*, 2010) and therefore, most guidelines on male infertility recommend performing at least two, or even three, sperm analyses

(WHO, 1999; NICE, 2004; NVOG, 2004; AUA and ASRM, 2006; Dohle *et al.*, 2007). In our cohort of 1223 men, 12 men (1.3%) switched from azoospermia to non-azoospermia or *vice versa* in one of their subsequent sperm analyses. Because this did not influence the prevalence of chromosomal abnormalities in the subgroups, and the number of cases in our cohort would have decreased substantially if we had included only men with two or more sperm analyses, we decided to include only the results of the first sperm analysis.

Azoospermia was significantly associated with chromosomal abnormalities, but we saw no correlation between sperm concentration or TMSC and chromosomal abnormalities in the non-azoospermic men. Sperm motility did show a positive association with chromosomal abnormalities. However, after analyzing the sperm concentration categories separately, it appeared that this association only existed in the severe oligozoospermia group (0–1 million/ml). It is therefore likely that the association found is explained by the measurement error of sperm motility in samples with very low concentrations (WHO, 2010).

In our retrospective study, hormone levels had been determined in fewer than half the cases. We found that FSH > 10 IU/l and LH > 12 IU/l were associated with chromosomal aberrations. Primary testicular failure is associated with high levels of gonadotrophins, and

Table II Sperm parameters and patient characteristics in a subgroup of 79 men with azoospermia in relation to chromosomal abnormalities.

Characteristic	n	Abnormal karyotype (12)	Normal karyotype (67)	Univariate analysis		
				OR	95% CI	P-value
Sperm volume per ml	79	4.1 (0.2–9.6)	3.2 (0.4–11)	1.25	0.969–1.61	0.085
Previous pregnancy by male partner	79	0	7.5%	0		0.999
FSH per IU/l	68	23.30 (4.6–48.4)	14.19 (0.29–49.8)	1.050	1.004–1.099	0.034
FSH > 10 IU/l	68	81.8%	52.6%	4.05	0.803–20.4	0.090
LH per IU/l	53	9.88 (2.5–25.3)	4.14 (0.1–21.3)	1.20	1.04–1.38	0.014
LH > 12 IU/l	53	33.3%	6.8%	6.83	1.11–42.0	0.038
Increased gonadotrophins ^a	69	81.8%	51.7%	4.20	0.834–21.1	0.082
Testosterone per nmol/l	64	15 (3.30–25)	16 (3.45–27)	0.95	0.84–1.1	0.35
Positive andrologic history ^b	77	50.0%	78.5%	0.275	0.077–0.984	0.047
Positive family history ^c	71	27.3%	25.0%	1.13	0.264–7.80	0.873
History of cryptorchidism	77	41.7%	26.2%	2.02	0.564–7.21	0.280

Data are median (range) or prevalence.

n, Number of cases of which data were available for analysis.

FSH, follicle-stimulating hormone; LH, luteinizing hormone.

^aIncreased gonadotrophins: FSH > 10 IU/l and/or LH > 12 IU/l.

^bPositive andrologic history: cryptorchidism (22), genital infection (5), vasectomy (3), orchidectomy (5), radiotherapy (1), chemotherapy (2), testicular biopsy (25), hypospadias or midline defect (2), varicocele (5), surgery for hydrocele (2), surgery for inguinal hernia (5), accidental testicular injury (1), testicular torsion (1), congenital anomaly of urogenital tract (12), exposition to diethylstilbestrol *in utero* (1). Note: several events may have occurred in one case.

^cPositive family history: (male) relatives with infertility (12), recurrent miscarriages (4), children with congenital anomalies or mental retardation (2).

Table III Sperm parameters and patient characteristics in a subgroup of 1144 non-azoospermic men applying for ICSI in relation to chromosomal abnormalities.

Characteristic	n	Abnormal karyotype (26)	Normal karyotype (1118)	Univariate analysis		
				OR	95% CI	P-value
Sperm volume per ml	1144	3.55 (1–13.9)	3.80 (0.1–15.1)	0.953	0.774–1.173	0.649
Sperm concentration per million/ml	1144	5.000 (0.0001–40)	6.000 (0.000003–185)	0.994	0.966–1.023	0.668
Sperm motility per %	1144	26.0 (0–100)	18.0 (0–100)	1.020	1.003–1.018	0.024
TMSC per million	1144	2.95000 (0–38.5)	2.84665 (0–466.2)	0.996	0.975–1.018	0.717
Previous pregnancy by male partner	1135	7.7%	21.4%	0.307	0.072–1.307	0.110
Previous miscarriage(s)	239	50%	34.6%	1.89	0.117–30.6	0.654
ICSI because of TFF	1144	15.4%	16.2%	0.941	0.321–2.76	0.912
FSH per IU/l	464	8.075 (3.33–29.4)	6.830 (0.19–46.6)	1.035	0.969–1.106	0.305
LH per IU/l	209	4.900 (1–10.2)	3.835 (0.09–25.1)	1.098	0.913–1.321	0.320
Increased gonadotrophins ^a	465	41.7%	32.2%	1.50	0.469–4.81	0.494
Testosterone per nmol/l	388	17 (6.5–25)	17 (5.8–51)	0.967	0.88–1.1	0.49
Positive andrologic history ^b	1114	30.8%	48.9%	0.464	0.200–1.08	0.074
Positive family history ^c	1046	20.0%	18.2%	1.12	0.416–3.03	0.820
History of cryptorchidism	1113	15.4%	20.1%	0.721	0.246–2.11	0.551

Data are median (range) or prevalence.

n, Number of cases of which data were available for analysis.

TMSC, total motile sperm count; FSH, follicle-stimulating hormone; LH, luteinizing hormone; TFF, total fertilization failure in previous IVF.

^aIncreased gonadotrophins: FSH > 10 IU/l and/or LH > 12 IU/l.

^bPositive andrologic history: cryptorchidism (223), genital infection (89), vasectomy (30), orchidectomy (44), radiotherapy (8), chemotherapy (16), testicular biopsy (6), hypospadias or midline defect (6), varicocele (117), spermatocele (5), surgery for hydrocele (6), surgery of urethra or ejaculatory duct (9), surgery for inguinal hernia (116), accidental testicular injury (28), testicular torsion (22), congenital anomaly of urogenital tract (1), exposition to diethylstilbestrol *in utero* (3). Note: several events may have occurred in one case.

^cPositive family history: (male) relatives with infertility (132), recurrent miscarriages (45), children with congenital anomalies or mental retardation (32).

the diagnosis of non-obstructive azoospermia is based on elevated FSH (NICE, 2004; Dohle *et al.*, 2007; Ferlin *et al.*, 2007; Kosar *et al.*, 2010; NVOG, 2010). No studies have been published in which the level of gonadotrophins was analyzed in azoospermic and non-azoospermic men separately for a correlation with chromosomal abnormalities. In our azoospermia group, men with high gonadotrophins had the highest prevalence of chromosomal abnormalities compared with men with normal gonadotrophin levels. FSH was positively associated with chromosomal abnormalities, although no statistically significant cut-off level could be determined due to the small number of cases in this subgroup. Future studies should confirm the association between gonadotrophin levels and chromosomal abnormalities in azoospermic men as a simple test to specify the risk of chromosomal abnormalities. In non-azoospermic men, a univariate regression analysis showed no association between FSH or LH and chromosomal abnormalities. Our subjects also showed a wide range of serum total testosterone and we found no association with chromosomal abnormalities.

Cryptorchidism had a prevalence of 21% in our cohort, and was almost evenly distributed in men with and without chromosomal abnormalities. Cryptorchidism showed no association with chromosomal abnormalities and our study does not confirm the hypothesis that cryptorchidism and infertility have a mutual chromosomal cause (Akre and Richiardi, 2009).

Of the 1223 subjects in our cohort, 20% had previously achieved conception, of which 71% had achieved at least one pregnancy with the current partner. These pregnancies were not realized by IVF or ICSI, but had occurred spontaneously or by intrauterine insemination. If there had been a previous pregnancy, whether it ended in a miscarriage, ectopic pregnancy, stillbirth, a child with congenital anomalies or a healthy newborn, the chance of finding a chromosomal abnormality was significantly decreased. This is in agreement with the observation that chromosomal abnormalities are predominantly found in azoospermic men, while previous pregnancies are mainly seen in the non-azoospermic group. In 79 azoospermic men, five reported their partners had had a previous pregnancy. Two of these men had undergone a vasectomy and one chemotherapy. It is most likely that the pregnancies occurred before the onset of azoospermia. We found no statistically significant association between previous pregnancy or miscarriage and chromosomal abnormalities in non-azoospermic men.

In the azoospermic men, a positive andrologic history (e.g. cryptorchidism, varicocele, genital infection, chemotherapy and vasectomy) lowered the risk of an aberrant karyotype (OR 0.28). Our data suggest that azoospermic men who have a plausible explanation for their azoospermia in their medical history have a lower risk of chromosomal abnormalities than azoospermic men with an uneventful medical history. The same was found in non-azoospermic men, although this association did not reach statistical significance.

All couples received an extensive questionnaire on their medical and family history before their first visit to our fertility centre. However, not all questions were filled in completely, which resulted in missing data. Moreover, due to the retrospective design of the study, we only had a limited number of sperm analyses and hormone results available. Furthermore, although the cohort consisted of a large group of infertile men, the prevalence of chromosomal abnormalities was low. A multivariate regression analysis was performed on all variables and chromosomal abnormalities, but it was

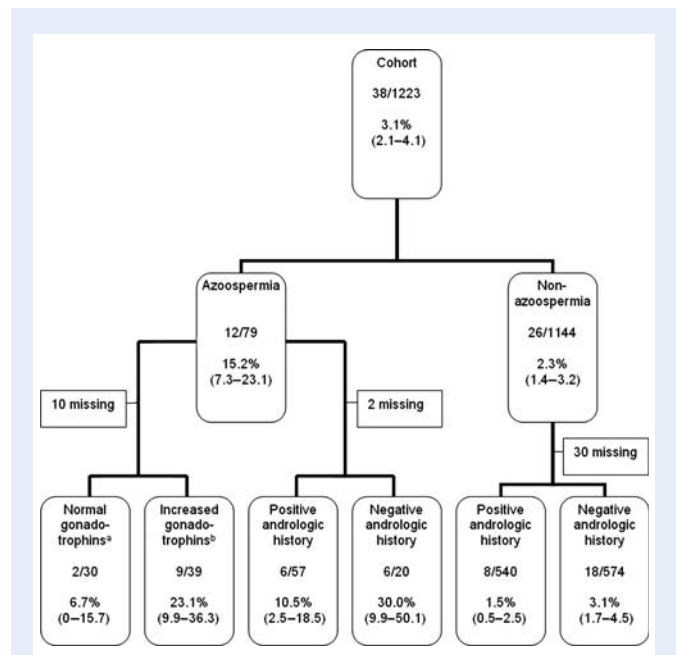


Figure 1 Prevalences (and 95% CIs) of chromosomal abnormalities in subgroups of men with azoospermia or applying for ICSI. ^aFSH \leq 10 IU/l and LH \leq 12 IU/l, ^bFSH > 10 IU/l and/or LH > 12 IU/l.

not conclusive due to the small sizes of the different subgroups. We therefore created a model, based on the univariate regression analysis and presented it in Fig. 1. This shows that azoospermic men with high gonadotrophin levels have the highest risk of carrying a chromosomal abnormality, while a possible explanation for poor sperm quality (i.e. a positive andrologic history) lowers this risk in azoospermic and non-azoospermic men. Whether these conclusions apply to all infertile men remains to be studied.

In conclusion, we show that in our cohort of infertile men, in whom the a priori risk of chromosomal abnormalities is 3.1% (CI 2.1–4.1), sperm concentration is not a good predictor for chromosomal abnormalities, although the total absence of spermatozoa is. Sperm volume and motility do not correlate with chromosomal abnormalities. If a man has previously achieved conception, the risk of a chromosomal abnormality significantly decreases (OR 0.21).

Azoospermic men had a prevalence of chromosomal abnormalities of 15.2% (CI 7.3–23.1). When gonadotrophin levels are increased (FSH > 10 IU/l and/or LH > 12 IU/l), the risk of a chromosomal abnormality increases significantly. In azoospermic men with a positive andrologic history, irrespective of the gonadotrophin levels, the risk decreases (OR 0.28).

In non-azoospermic men eligible for ICSI, the prevalence of chromosomal abnormalities was 2.3% (CI 1.4–3.2), and none of the parameters studied provides a better risk assessment for chromosomal abnormalities in this group of men.

The findings of our study allow for a more specific risk estimate on chromosomal abnormalities in subgroups of infertile men. The recommendation on who should be offered karyotyping should be based on cost-effectiveness studies, in which costs of adverse events due to chromosomal abnormalities in men (e.g. miscarriages and children

with congenital anomalies) should be included. The data obtained in our cohort can be used in these future economic models.

Authors' roles

E.C.D. helped design the study, collected and analyzed the data and wrote the manuscript. H.G. contributed to the statistical analysis. C.M.A.v.R.-A. helped design the study and provided the cytogenetic data. T.D. was responsible for the chromosomal analyses. J.v.E.-A. was responsible for the sperm analyses. J.A.L. was responsible for the design of the study. All authors revised the manuscript.

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